PUBLIC HEALTH SERVICE
U.S. FOOD AND DRUG ADMINISTRATION
OFFICE OF FOOD SAFETY
SHELLFISH AND AQUACULTURE POLICY BRANCH
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SHELLFISH LABORATORY EVALUATION CHECKLIST
PCR Microbiology

LABORATORY:		
ADDRESS:		
TELEPHONE:	**FAX:**	
EMAIL:		
DATE OF EVALUATION:	**DATE OF REPORT:**	**LAST EVALUATION:**

LABORATORY REPRESENTED BY:	**TITLE:**
LABORATORY EVALUATION OFFICER:	**SHELLFISH SPECIALIST:**
OTHER OFFICIALS PRESENT:	**TITLE:**

Items which do not conform are noted by: **Conformity is noted by a "√"**

C- Critical K – Key O – Other N/A- Not Applicable

Check the applicable analytical methods:

	MPN Real-time PCR method for *Vibrio vulnificus* detection in Oysters [PART III] SmartCycler II and AB 7500 Fast
	MPN Real-time PCR method for *Vibrio parahaemolyticus* detection in Oysters [PART III] SmartCycler II and AB 7500 Fast

PART I – QUALITY ASSURANCE				
CODE	**REF**	**ITEM**		
		1.1 Quality Assurance (QA) Plan		
K	4, 6		1.1.1	Written Plan (Check √ those items which apply).
				a. Organization of the Laboratory.
				b. Staff training requirements.
				c. Standard operating procedures (SOPs).
				d. Internal quality control measures for equipment, their calibration maintenance, repair, performance and rejection criteria established.
				e. Laboratory safety.
				f. Internal performance assessment.
				g. External performance assessment.
C	**4**		**1.1.2**	**The QA plan is implemented.**
K	6		1.1.3	The Laboratory participates in a proficiency testing program annually. Specify the program(s): _____
		1.2 Educational/Experience Requirements		
C	**State's Human Resources Department**		**1.2.1**	**In state/county laboratories, the supervisor must have at least a bachelor's degree in microbiology, biology or equivalent discipline with at least two years of laboratory experience.**
K	State's Human Resources Department		1.2.2	In state/county laboratories, the analysts meet the state/county educational and experience requirements for processing samples in a public health laboratory.
C	**USDA Microbiology & EELAP**		**1.2.3**	**In commercial laboratories, the supervisor must have at least a bachelor's degree in microbiology, biology or equivalent discipline with at least two years of laboratory experience.**
K	USDA Microbiology & EELAP		1.2.4	In commercial laboratories, the analysts must have at least a high school diploma and at least three months of experience in laboratory sciences.
		1.3 Work Area		
O	4, 6		1.3.1	Adequate for workload and storage.
K	6		1.3.2	Clean, well lighted.
K	6		1.3.3	Adequate temperature control.
O	6		1.3.4	All work surfaces are nonporous, easily cleaned and disinfected.
K	6		1.3.5	Microbiological quality of the air contains fewer than 15 colonies/plate for a 15 minute exposure determined monthly. The results are recorded and records maintained.
		1.4 Laboratory Equipment		
K	5		1.4.1	To determine the pH of prepared media and reagents, the pH meter has a standard accuracy of 0.1 pH units.
K	9		1.4.2	pH electrodes consisting of pH half-cell and reference half-cell or equivalent combination electrode free from (Ag/AgCl) or contains an ion exchange barrier preventing passage of Ag ions into the medium which may affect the accuracy of the pH reading.
K	6		1.4.3	The effect of temperature on the pH is compensated for by an internal/external ATC probe or by manual adjustment (*Circle the appropriate type of adjustment*).
K	4		1.4.4	The pH meter is calibrated daily or with each use as per product literature. Results are recorded and records maintained.

K	6		1.4.5	A minimum of two standard buffer solutions are used to calibrate the pH meter. The first is near the electrode isopotential point (pH 7). The second is near the expected sample pH (i.e. pH 4 or pH 10). Standard buffer solutions are used once and discarded.
O	4		1.4.6	Electrode acceptability is determined daily or with each use by the millivolt procedure or through determination of the slope (*Circle the method used*).
K	5		1.4.7	The balances used provide a sensitivity of at least 0.1 g at the weights of use.
K	6		1.4.8	Balance calibrations are checked monthly according to manufacturer's specifications using NIST Class S or ASTM Class 1 or 2 weights or equivalent. The accuracy of the balance is verified at the weight range of use. Results are recorded and records maintained.
K	6		1.4.9	Refrigerator temperatures are monitored at least once daily on workdays. Results are recorded and records maintained.
K	1		1.4.10	Refrigerator temperatures are maintained between 0 and 4 °C, except for reagent refrigerators which are maintained between 2 and 8 °C.
C	**7**		**1.4.11**	**Freezer temperature is maintained at -15 °C or below.**
O	7		1.4.12	Freezer temperature is monitored at least once daily on workdays. Results are recorded and records maintained.
C	**5**		**1.4.13**	**The temperature of the incubator is maintained at 35 ± 2.0 °C.**
K	6		1.4.14	Thermometers used in the air incubators are graduated at no greater than 0.5°C increments.
K	5		1.4.15	Working thermometers are located on top and bottom shelves of use in the air incubator or appropriately placed based on the results of spatial temperature checks.
K	4, 6		1.4.16	Air incubator temperatures are taken twice daily on workdays. Results are recorded and records maintained.
C	**3**		**1.4.17**	**All working thermometers are appropriately immersed.**
C	**2, 20**		**1.4.18**	**Working thermometers are either: calibrated mercury-in-glass thermometers, calibrated non-mercury-in-glass thermometers, or appropriately calibrated electronic devices, including Resistance Temperature Devises (RTDs) and Platinum Resistance Devices (PTDs) with an accuracy and tolerance appropriate for the application.**
C	**6, 20**		**1.4.19**	**A standards thermometer has been calibrated by NIST or a qualified calibration laboratory using a primary standard traceable to NIST or an equivalent authority at the points 0 and 35. These calibration records are maintained.**
K	3, 5		1.4.20	Standard thermometers are checked annually for accuracy by ice point determination. Results are recorded and maintained. Date of most recent determination: _____
C	**2, 20**		**1.4.21**	**Either mercury-in-glass thermometers, non-mercury-in-glass thermometers having the accuracy (uncertainty), tolerance and response time of mercury or low drift electronic resistance thermometers with an accuracy of ≤0.05 °C are used as the laboratory standards thermometer (*Circle the thermometer type used*).**
K	3, 8		1.4.22	All working thermometers are checked annually against the standards thermometer at temperature(s) of use. Results are recorded and records maintained.
O	6		1.4.23	Appropriate pipet aids are available and used to inoculate samples.
K	2		1.4.24	Micropipettors are calibrated annually at appropriate volumes used and checked for accuracy quarterly. Results are recorded and records maintained.
		1.5 Labware and Glassware Washing		
K	5		1.5.1	Utensils, containers, glassware and plasticware are clean borosilicate glass, stainless steel or other noncorroding material.
K	5		1.5.2	Culture tubes are new and of a suitable size to accommodate the volume for nutritive ingredients and sample.
K	5		1.5.3	Dilution bottles and tubes are made of borosilicate glass or plastic and closed with secure caps or screw caps with nontoxic liners.

K	5		1.5.4	Graduations are indelibly marked on dilution bottles and tubes or an acceptable alternative method is used to ensure appropriate volumes.
K	5		1.5.5	In washing reusable pipets, glassware and labware, a succession of at least three fresh water rinses plus a final rinse of deionized water is used to thoroughly rinse off all detergent.
C	**2**		**1.5.6**	**An alkaline or acidic detergent is used for washing glassware/labware.**
C	**6**		**1.5.7**	**With each load of labware/glassware washed, the contact surface of several dry pieces from each load are tested for residual detergent (acid or alkali as appropriate) with aqueous 0.04% bromothymol blue (BTB) solution. Results are recorded and records maintained.**
		1.6 Sterilization and Decontamination		
K	5		1.6.1	The autoclave is of sufficient size to accommodate the workload.
K	4		1.6.2	Routine autoclave maintenance is performed and the records maintained.
C	**6, 20**		**1.6.3**	**The autoclave provides a sterilizing temperature of 121 ± 2 °C as determined for each load using a calibrated maximum registering thermometer. As an alternative, an appropriate temperature monitoring device is used in place of the maximum registering thermometer when these are unavailable due to the ban on mercury.**
K	6		1.6.4	An autoclave standards thermometer has been calibrated by a qualified calibration laboratory using a primary standard traceable to NIST or an equivalent authority at 121 °C. Calibration at 100 °C, the steam point is also recommended but not required.
K	10		1.6.5	The autoclave standards thermometer is checked every five years for accuracy at either 121 °C or at 100 °C, the steam point if the thermometer has been previously calibrated at this temperature. Date of most recent determination: _____
K	1		1.6.6	Working autoclave thermometers are checked against the autoclave standards thermometer at 121 °C yearly. Date of last check:_____
K	6		1.6.7	Spore strips/suspensions appropriate for use in an autoclave media cycle are used monthly according to manufacturer's instructions to evaluate the effectiveness of the sterilization process. Results are recorded and the records maintained.
O	6		1.6.8	Heat sensitive tape is used with each autoclave batch.
K	6		1.6.9	Autoclave sterilization records including length of sterilization, total heat exposure time and chamber temperature are maintained. Type of record: Autoclave log, computer printout or chart recorder tracings (*Circle the appropriate type or types*).
K	6		1.6.10	For dry heat sterilized material, the hot-air sterilizing oven provides heating and sterilizing temperatures in the range of 160 to 180 °C.
K	5		1.6.11	A thermometer capable of determining temperatures accurately in the range of 160 to 180 °C is used to monitor the operation of the hot air sterilizing oven.
K	8		1.6.12	Records of temperature and exposure times are maintained for the operation of the hot-air sterilizing oven.
K	6		1.6.13	Spore strips/suspensions appropriate for use in dry heat are used quarterly to evaluate the effectiveness of the sterilization process in the hot-air oven. Results are recorded and records maintained.
K	5		1.6.14	Reusable pipets are stored and sterilized in aluminum or stainless steel containers.
K	5		1.6.15	Reusable pipets (in canisters) are sterilized in a hot-air oven at 170 °C for 2 hours.
C	**2**		**1.6.16**	**The sterility of reusable pipets is determined with each load sterilized. Results are recorded and records maintained.**

C	**2**		**1.6.17**	**The sterility of autoclave sterilized disposable pipet tips and microcentrifuge tubes is determined with each load sterilized. Results are recorded and records maintained.** **If presterilized pipet tips and microcentrifuge tubes are purchased certificate should be maintained and sterility confirmed as in 1.6.18.**
C	**2**		**1.6.18**	**The sterility of presterilized disposable pipets, pipet tips and microcentrifuge tubes is determined with each lot received. Results are recorded and records maintained.**
K	8		1.6.19	Spent broth cultures and agar plates are properly decontaminated before disposal.
		1.7 Media Preparation		
K	13, 14		1.7.1	Alkaline peptone water (APW) is prepared from the individual components and pH adjusted appropriately.
K	6		1.7.2	Media components are properly stored in a cool dry place.
O	6		1.7.3	Media components are labeled with the analyst's initials, date of receipt and date opened.
O	6		1.7.4	Dehydrated media are labeled with date of receipt and date opened.
C	**6**		**1.7.5**	**Caked or expired media or media components are discarded.**
C	**6**		**1.7.6**	**Reagent water for media and diluent preparation is analyzed for residual chlorine monthly and is at a non-detectable level (≤0.1 ppm). Results are recorded and records maintained**
K	6		1.7.7	Reagent water for media and diluent preparation contains <100 CFU/mL as determined monthly using the heterotrophic plate count method. Results are recorded and records maintained.
K	5		1.7.8	The volume and concentration of media in the tube is suitable for the amount of sample inoculated.
C	**6**		**1.7.9**	**Media broths are not in the autoclave for more than 60 minutes.**
C	**1**		**1.7.10**	**Media and diluent sterility is determined for each load sterilized. Results are recorded and records maintained.**
C	**1**		**1.7.11**	**Media productivity is determined using media-appropriate positive and negative control cultures for each lot of dehydrated media received or with each batch of media prepared when the medium is made from its individual components.**
C	**6**		**1.7.12**	**The pH of the prepared media is determined after sterilization to ensure that it is consistent with manufacturer requirements and/or method tolerance. Results are recorded and records are maintained.**
		1.8 Storage of Prepared Culture Media		
K	5		1.8.1	Prepared culture media are stored in a cool, clean, dry place where excessive evaporation and the danger of contamination is minimized.
K	8		1.8.2	Stored media are labeled with the storage expiration date or sterilization date.
K	5		1.8.3	Storage of prepared culture media at room temperature does not exceed 7 days.
K	2		1.8.4	Storage under refrigeration of prepared broth media with loose fitting closures does not exceed 1 month.
K	6		1.8.5	Storage under refrigeration of prepared culture media with screw- cap closures does not exceed 3 months.
K	11		1.8.6	All prepared broth media stored under refrigeration is warmed to room temperature prior to use, without exceeding incubation temperature.
PART II – SAMPLES				
		2.1 Sample Collection, Transportation and Receipt		
C	**2, 6**		**2.1.1**	**A representative sample is collected and a chain of custody documenting the history of the sample(s) from collection to final disposal has been established.**
K	5		2.1.2	Shellfish samples as received are collected in clean, waterproof, puncture resistant containers loosely sealed or are rejected for regulatory analysis.

K	5		2.1.3	Shellfish samples as received are labeled with the collector's (or if PHP, company/processor and collector's) name, the source, the time and date of collection or are rejected for regulatory analysis.
C	**5**		**2.1.4**	**Immediately after collection, shellfish samples are placed in dry storage (ice chest or equivalent) which is maintained between 2 and 10 °C with ice or cold packs for transport to the laboratory. Once received, the samples are placed under refrigeration unless processed immediately.**
C	**1**		**2.1.5**	**Analysis of the samples is initiated as soon as possible after collection, but not to exceed 36 h. If processing IQF samples, samples are defrosted under refrigeration for no longer than 36 h once removed from the freezer.**
		2.2 Preparation of Samples for Analysis		
K	2, 6		2.2.1	Shucking knives, scrub brushes and blender jars are autoclave sterilized for 15 minutes.
O	2		2.2.2	Blades of shucking knives are not corroded.
K	5		2.2.3	The hands of the analyst are thoroughly washed with soap and water or new gloves are donned, immediately prior to cleaning the shells of debris.
O	2		2.2.4	The faucet used for rinsing the shellfish does not contain an aerator.
K	5		2.2.5	Shellfish are scrubbed with a stiff, sterile brush and rinsed under tap water of drinking water quality.
K	5		2.2.6	Samples are allowed to drain in a clean container or on clean towels prior to opening
K	5, 15		2.2.7	Immediately prior to shucking, the hands or gloved hands of the analyst are thoroughly washed with soap and water and rinsed in 70% alcohol. The gloves if worn are latex, nitrile and/or stainless steel mesh to protect analyst's hands from injury.
C	**5**		**2.2.8**	**Shellfish are not shucked through the hinge.**
C	**5**		**2.2.9**	**The contents of the sample (liquor and meat) are shucked into a sterile, tared blender jar or other sterile container.**
C	**5**		**2.2.10**	**A representative sample of at least 12 shellfish is used for analysis**
C	**2, 5**		**2.2.11**	**A quantity of meat and liquor is sufficient to cover the blender blades or additional oysters are used in order to ensure sample homogeneity.**
K	2, 13		2.2.12	The sample can be processed directly or a 1:1 dilution of shellfish:diluent made. If a dilution is made, the sample is weighed to the nearest 0.1 g and an equal amount, by weight, of diluent is added.
K	13		2.2.13	Sterile phosphate buffered saline (pH 7.4) is used as the sample diluent.
C	**5**		**2.2.14**	**Samples are blended for 60 to 120 seconds until homogenous.**
PART III- PCR METHOD FOR VIBRIO VULNIFICUS AND VIBRIO PARAHAEMOLYTICUS DETECTION IN OYSTERS				
		3.1 APW Enrichment		
K	5		3.1.1	Sterile phosphate buffered saline (PBS) is used as the sample diluent.
C	**5, 15**		**3.1.2**	**The 1:10 dilution is prepared gravimetrically with PBS. All successive dilutions are prepared volumetrically.** **For example, if an initial 1:1 dilution of the sample was used for blending, the 1:10 dilution is prepared by adding 20 g of sample homogenate to 80 ml of PBS. If the homogenate was not diluted, the 1:10 dilution is prepared by adding 10 g of sample homogenate to 90 ml of PBS.**
C	**17**		**3.1.3**	**Appropriate sample dilutions are inoculated into APW.** **Specify dilution(s) used _____** **Specify number of tubes per dilution _____**

C	**2, 15**		**3.1.4**	**For *V. parahaemolyticus* analysis, a tdh+, trh+ *V. parahaemolyticus* culture diluted to <10^3 per ml is used as a positive process control. A non *V. parahaemolyticus* culture is used as a negative process control.** **For *V. vulnificus* analysis, a *V. vulnificus* culture diluted to <10^3 per ml is used as a positive process control. A non *V. vulnificus* culture is used as a negative process control.** **The process control cultures accompany the samples throughout incubation, isolation, and confirmation. Records are maintained.**
C	**13**		**3.1.5**	**Inoculated APW enrichment tubes are incubated at 35 +/- 2 °C.**
C	**13**		**3.1.6**	**Tubes are read after 18 – 24 hours of incubation. Clear tubes are negative. Turbid tubes are positive and shall be further processed.**
		3.2 PCR Reagents		
C	**14, 15**		**3.2.1**	**Lyophilized primers and probes are stored according to manufacturer's instructions.**
K	14, 15		3.2.2	Fluorescent probes are stored in light occluding tubes or containers.
C	**14, 15, 18, 19**		**3.2.3**	**The PCR forward and reverse primers and probes are appropriate for the platform.** **<u>For Total and Pathogenic Vp Real-time PCR Method</u>** tdh_269-20: 6FAM-5'-TGACATCCTACATGACTGTG-3'-MGBNFQ trh_133-23: NED/TET-5'-AGAAATACAACAATCAAAACTGA-3'-MGBNFQ tlh_1043: JOE/TEXAS RED-5'- CGCTCGCGTTCACGAAACCGT-3'-BHQ2 IAC_109: CY5-5'- TCTCATGCGTCTCCCTGGTGAATGTG-3'- BHQ2 trh_20F: 5'-TTGCTTTCAGTTTGCTATTGGCT-3' trh_292R: 5'-TGTTTACCGTCATATAGGCGCTT-3' tdh_89F: 5'-TCCCTTTTCCTGCCCCC-3' tdh_321R: 5'-CGCTGCCATTGTATAGTCTTTATC-3' tlh_884F: 5'-ACTCAACACAAGAAGAGATCGACAA-3' tlh_1091R: 5'-GATGAGCGGTTGATGTCAAA-3' IAC_46F: 5'-GACATCGATATGGGTGCCG-3' IAC_186R: 5'-CGAGACGATGCAGCCATTC-3' **<u>For Vv Real-time PCR Method</u>** vvhF: 5'-TGTTTATGGTGAGAACGGTGACA-3' vvhR: 5'-TTCTTTATCTAGGCCCCAAACTTG-3' vvh Probe: Cy5-5'-CCGTTAACCGAACCACCCGCAA-3'-IAbRQ IAC 46F: 5'-GACATCGATATGGGTGCCG-3' IAC 186R: 5'-CGAGACGATGCAGCCATTC-3' IC Probe: JOE-5'-TCTCATGCGTCTCCCTGGTGAATGTG-3'-IABkFQ
C	**14, 18**		**3.2.4**	**Lyophilized forward and reverse primers, and probes, are hydrated with TE buffer to produce a 0.1 mM stock solution.**
C	**14, 18**		**3.2.5**	**Using molecular grade, nuclease free water, primer and probe stock solutions are diluted to produce a 0.01 mM working solution.**
C	**14, 18**		**3.2.6**	**Reconstituted primers and probes are stored in a -20 °C manual defrost freezer for up to 5 freeze thaw cycles, not to exceed two years.**
C	**21, 22**		**3.2.7**	**Platinum *Taq* DNA is stored in -20 °C manual defrost freezer until first use. After first use, can be stored between 2-8 °C.**
C	**21, 22**		**3.2.8**	**PCR reagents (dNTPs, buffer, MgCl2, fluorescent dyes) are stored in -20°C manual defrost freezer until first use. After first use, they can be stored between 2-8 °C.**
		3.3 DNA Extraction		
C	**14, 18**		**3.3.1**	**All microcentrifuge tubes and pipet tips are sterile.**

C	**14, 18**		**3.3.2**	**Pipet tips have aerosol barriers.**
K	14, 18		3.3.3	Latex or nitrile gloves are worn throughout the extraction and PCR preparation process.
K	14, 18		3.3.4	All work surfaces, centrifuge racks and equipment used in PCR analysis are disinfected immediately prior to DNA extraction, Master Mix preparation and PCR analysis.
C	**14, 18**		**3.3.5**	**Aseptic technique is observed throughout the extraction and PCR analysis.**
C	**14, 18**		**3.3.6**	**One thousand (1000) µL aliquots from each positive APW enrichment tube, including the process controls, are extracted.**
C	**14, 18**		**3.3.7**	**Positive APW aliquots are placed in sterile microcentrifuge tubes and heated at 95-100 °C for 10 minutes.**
K	14, 18		3.3.8	A set of positive and negative process controls are included with each batch of samples in a heating block/boiling bath.
C	**14, 18**		**3.3.9**	**After boiling, tubes are chilled in ice or immediately frozen in a manual defrost freezer for future analysis. Boil preps may be refrigerated not to exceed 72 hours.**
K	14, 18		3.3.10 Frozen extracts are analyzed within 6 months of frozen storage.	
		3.4 Preparation of the Master Mix for PCR		
C	**14, 16, 18**		**3.4.1**	**Nuclease-free microcentrifuge tubes and pipette tips, with filters, are used in Master Mix preparation.**
C	**14, 16, 18**		**3.4.2**	**For each reaction, add the specified amount of water, buffer, MgCl2, dNTPs, specific primers, nuclease probes, *Taq*, and internal control DNA is added.**
K	14, 21, 18		3.4.3	The Master Mix is gently vortexed to mix constituents and then briefly spun.
C	**14, 16, 18**		**3.4.4**	**Twenty-three (23) µL of Master Mix is used for each PCR reaction.**
C	**14, 16, 18**		**3.4.5**	**Master Mix must be used on the day of preparation or stored at –20 °C until time of use.**
		3.5 PCR		
C	**14, 19**		**3.5.1**	**If previously frozen, the DNA extracts are completely thawed at temperatures no warmer than room temperature. Immediately prior to use, DNA extracts are centrifuged at >5,000 x g for 2 minutes to remove particulate matter and cell debris.**
C	**14, 19**		**3.5.2**	**Two (2) µL of DNA template is added to each reaction tube or plate well containing 23 µL of Master Mix for a total PCR reaction volume of 25 µL.**
K	14, 19		3.5.3	Two (2) µL of molecular grade, nuclease free water is added to a reaction tube or plate well containing 23 µL of Master Mix for each batch of Master Mix prepared as a no template control.
C	**14, 19**		**3.5.4**	**Two (2) µL of DNA template extracted from the negative process control culture is added to a reaction tube or plate well containing 23 µL of Master Mix.**
C	**14, 19**		**3.5.5**	**Two (2) µL of DNA template extracted from the positive process control culture is added to a reaction tube or plate well containing 23 µL of Master Mix**.
O	14, 19		3.5.6	Two (2) µL of DNA template extracted from the positive control culture (prepared separately from the positive process control) is added to a reaction tube or plate well containing 23 µL of Master Mix as the positive PCR control.
K	14, 19		3.5.7	Immediately prior to loading the reaction tubes or plates into the instrument they are centrifuged for 3-5 seconds to ensure that all reagents and the DNA template are in the bottom of the tube to optimize the PCR amplification process.
C	**16**		**3.5.8**	**After centrifugation, tubes or plates are inserted into the instrument.**
		3.6 PCR Amplification		
C	**14, 19**		**3.6.1**	**The appropriate instrument platform is used for the protocol.**
K	16		3.6.2	Manufacturer's instructions are followed in operating the instrument.
C	**14, 19**		**3.6.3**	**The PCR cycle parameters used are appropriate for the protocol.**
K	14, 19		3.6.4	Optical calibrations for the dyes being used are current, per the instrument manufacturer's recommendations.

C	**14, 19**		**3.6.5**	**The analysis settings are adjusted as specified in the protocol.**
		3.7 Computation of Results		
K	14, 19		3.7.1	All runs in which the positive control generates a Ct value for the target(s) of interest and the negative control reaction generates no Ct value for the target(s), but a Ct value for the internal control are considered valid.
C	**2**		**3.7.2**	**Data is quality checked by the analyst.**
C	**14, 19**		**3.7.3**	**All reactions in a valid run which generate a Ct value for the target(s) of interest with a sigmoidal amplification curve are considered to be positive.**
C	**16**		**3.7.4**	**Any sample which does not demonstrate a sigmoidal amplification curve may have a reported positive/negative determination that is discrepant from the instrument if appropriately justified using the raw fluorescent data.**
K	16		3.7.5	All reactions in a valid run which do not generate a Ct value for the target(s) of interest, but do generate a Ct value for the internal control are considered negative.
C	**16**		**3.7.6**	**Any reaction in which no Ct value is generated for the target(s) of interest or the internal control is considered invalid and should be re-tested.**
C	**13**		**3.7.7**	**Upon determination of positive reactions, refer to the original positive dilutions of APW and record MPN values as derived from the calculator in Appendix 2 of the FDA Bacteriological Analytical Manual (BAM).**
K	13		3.7.8	For APW enrichment, results are reported as MPN/g of sample.

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LABORATORY:			DATE of EVALUATION:
SHELLFISH LABORATORY EVALUATION CHECKLIST **SUMMARY OF NONCONFORMITIES**			
Page	Item	Observation	Documentation Required

LABORATORY STATUS	
LABORATORY	**DATE**
LABORATORY REPRESENTATIVE:	
PCR MICROBIOLOGY COMPONENT: (Part I-III)	
A. Results Total # of Critical (C) Nonconformities in Parts I-III Total # of Key (K) Nonconformities in Parts I-III Total # of Critical (C), Key (K), and Other (O) Nonconformities in Parts I-III	 _____ _____ _____

B. Criteria for Determining Laboratory Status of the PCR Microbiology Component:

1. **Does Not Conform Status**: The PCR Microbiological component of this laboratory is not in conformity with NSSP requirements if:
   a. The total # of Critical nonconformities is **≥4 or**
   b. The total # of Key nonconformities is **≥13 or**
   c. The total # of Critical, Key and Other is **≥18**
2. **Provisionally Conforms Status**: The PCR microbiological component of this laboratory is determined to be provisionally conforming to NSSP requirements if the number of critical nonconformities is **≥ 1 but ≤ 3**.

C. Laboratory Status (*circle appropriate*)

Does Not Conform **Provisionally Conforms** **Conforms**

Acknowledgment by Laboratory Director/Supervisor:

All corrective Action will be implemented and verifying substantiating documentation received by the Laboratory Evaluation Officer on or before _____.

Laboratory Signature:_____ Date:_____

LEO Signature:_____ Date:_____

NSSP Form *6 – PCR Microbiology Checklist*, Rev. June 2024